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Potential mechanism of acute stent thrombosis with bivalirudin following percutaneous coronary intervention in acute coronary syndromes

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ABSTRACT

Background: Clinical trials have demonstrated an excess of acute stent thrombosis (AST) in acute coronary syndromes patients (ACS) undergoing percutaneous coronary intervention (PCI) with bivalirudin compared to heparin. We aimed to investigate the potential mechanisms responsible for thrombus formation under bivalirudin.

Methods: We compared heparin and bivalirudin during PCI for ACS in a prospective monocentre randomized study. Twenty patients were included after coronary angiography and received a loading dose (LD) of 180 mg of ticagrelor at the time of PCI. They were randomly assigned to heparin (70 UI/kg) intra-venous (IV) bolus or bivalirudin IV bolus of 0.75 mg/kg followed by an infusion of 1.75 mg/kg/h until the end of the PCI. The VASP index and thrombin generation test were used to assess the course of platelet reactivity (PR) and thrombin generation.

Results: Thrombin generation and PR were identical in both groups at baseline. There was no difference in the course of PR following the LD over time. An optimal PR inhibition was reached 4 h after the LD of ticagrelor. Heparin and bivalirudin infusion effectively inhibited thrombin generation during PCI. However, 4 h after the end of bivalirudin infusion, thrombin generation had returned to its baseline value whereas in the heparin group it remained significantly inhibited compared to baseline and to the bivalirudin group 4 h after the end of the infusion ($p < 0.01$ and $p < 0.02$ respectively).

Conclusions: The present study suggests that the short half-life of bivalirudin and the quick restoration of thrombin activity at a time when optimal PR is not reached may be responsible for acute stent thrombosis.
Clinicaltrial.gov: NCT02428725.

1. Introduction

Bivalirudin is a direct thrombin inhibitor, which can be used during percutaneous coronary interventions (PCI) in the setting of acute coronary syndrome (ACS) [1]. Several trials have demonstrated a net clinical benefit of bivalirudin compared to heparin with and without glycoprotein (GP) 2b/3a inhibitors [2–6]. In fact, bivalirudin significantly decreases major bleedings while being as efficient as heparin regarding ischemic endpoints [2–6]. However, bivalirudin use was associated with an excess early risk of acute stent thrombosis (AST) [2–5]. The mechanism responsible for such excess of AST remains controversial. Acute stent thrombosis is a multi-factorial process involving thrombin and platelets activation [7]. The care of ACS is based on the dual inhibition of platelet activity and thrombin generation to prevent further thrombus growth [1,8]. The role of fast acting and potent antiplatelet and anti-thrombin agents is therefore critical in particular in the setting of PCI. Of importance, it was demonstrated that the level of P2Y12-ADP receptor blockade necessary to prevent stent thrombosis is delayed for 2 to 6 h after the LD in ACS [9,10]. The current guidelines recommend

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discontinuing anticoagulation after revascularization [1]. Therefore following PCI in ACS, there may be a period when platelets are still activated and thrombin generation not adequately inhibited thus potentially promoting AST. In addition bivalirudin has a shorter half life than heparin which may increase the duration of this vulnerable period [11]. We thus compared the course of P2Y12-ADP receptor inhibition and thrombin generation in Non-ST elevation ACS (NSTE-ACS) patients undergoing PCI with heparin or bivalirudin.

2. Methods

We performed a monocentre prospective randomized controlled open-label study. Consecutives non-ST elevation ACS patients were eligible. Only intermediate and high risk ACS patients according to ESC guidelines were enrolled [1]. They were included after coronary angiography if a PCI was necessary. This study was a pre-specified sub-study of a larger clinical trial: NCT02428725. Informed consents were obtained for all patients before inclusion. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. The local ethic committee of our institution approved the study. All patients were naive of P2Y12-ADP receptor blockers and anticoagulant at the time of inclusion. No pretreatment was used.

PCI was performed through the radial route in all but one patient. All implanted stents were drug-eluting stents.

2.1. Antiplatelet and anticoagulant

Patients were randomized to heparin or bivalirudin using a computerized generated randomization list (1:1). All patients received an intra-venous bolus of aspirin 250 mg and a loading dose (LD) of 180 mg of ticagrelor at the time of PCI. The maintenance dose (MD) of aspirin was 75 mg and the MD of ticagrelor was 90 mg bi-daily in all patients [1].

In the heparin group patients received an intra-venous (IV) bolus of 70 UI/kg of unfractionated heparin (UFH) as soon as PCI was started. No further bolus was given. Routine measurements of activated clotting time were not performed. In the bivalirudin group, patients received an IV bolus of 0.75 mg/kg, as soon as PCI was started, immediately followed by an IV infusion of 1.75 mg/kg per hour. The infusion was stopped at the end of the PCI [1].

Exclusion criteria were: anticoagulant therapy, severe kidney failure (creatinin clearance <30 ml/min), hemodialysis, contra-indication to ticagrelor, bleeding disorder, cardiogenic shock, cardiac arrest, contra-indication to antiplatelet therapy, ongoing therapy with P2Y12-ADP blockers, platelet count <100G/L, history of bleeding diathesis, history of stroke, recent surgery (<1 month), use of medication with known interference with ticagrelor, bradycardia, history of heparin induced thrombocytopenia, glycoprotein 2b/3a inhibitors use.

2.2. Blood samples

Blood samples for VASP index analysis and thrombin generation assay were drawn by atraumatic venipuncture of the antecubital vein. The initial blood drawn was discarded to avoid measuring platelet activation induced by needle puncture; blood was collected into a Vacutainer containing 3.8% trisodium citrate and filled to capacity. The Vacutainer was inverted 3 to 5 times for gentle mixing and sent immediately to the hemostasis laboratory. For the thrombin generation assay, the citrated blood was double centrifuged at 2500*g* for 15 min at room temperature to obtain platelet-poor plasma. Plasma aliquots were stored at −80 °C until analysis.

2.3. Thrombin generation

Thrombin generation was studied using the Calibrated Automated Thrombogram assay (CAT®, Diagnostica Stago, Asnieres, France) according to manufacturers' instructions. Briefly, 80 μl of PPP were added to 20 μl of PPP reagent 5 pM® (Thrombinoscope b.v., Maastricht, the Netherlands), that is a mixture of tissue factor (5 pM final concentration in plasma) and phospholipids (4 μM final concentration in plasma). In a second well, PPP reagent 5 pM® was replaced with the same volume of Thrombin Calibrator® (Thrombinoscope b.v., Maastricht, the Netherlands) to correct thrombin generation curves for substrate consumption and inner filter fluorescence effects. Thrombin generation was triggered with 20 μl solution containing CaCl2 (16.7 mM final concentration) and the fluorogenic substrate Z-Gly-Gly-Arg-AMC (417 μM final concentration). Fluorescence was measured with fluorometer (Fluoroscan Ascent®, ThermoLabsystems, Helsinki, Finland). Acquisition of thrombin generation parameters was done with the appropriate software (Calibrated Automated Thrombogram® bv, Maastricht, The Netherlands). The following parameters of thrombogram were analyzed: the lag-time of thrombin generation, the time to reach the peak of thrombin (time to Peak), the thrombin peak (Peak), the endogenous thrombin potential (ETP) that reflects the total amount of thrombin activity [12]. Normal values were defined on a cohort of 30 healthy control subjects. Healthy control subjects were normal individuals matched for the main demographic characteristic.

2.4. P2Y12-ADP receptor blockade

PR was measured using the VASP index. Blood samples for VASP index analysis were drawn by atraumatic venipuncture of the antecubital vein. The initial blood drawn was discarded to avoid measuring platelet activation induced by needle puncture; blood was collected into a Vacutainer containing 3.8% trisodium citrate and filled to capacity. The Vacutainer was inverted 3 to 5 times for gentle mixing and sent immediately to the hemostasis laboratory. VASP index phosphorylation analysis was performed within 24 h of blood collection by an experienced investigator using the CY-QUANT VASP/P2Y12 enzyme-linked immunosorbent assay (Biocytex, Marseille, France) [13]. Briefly, after a first step of parallel whole blood sample activation with PGE1 and PGE1 + ADP, platelets from the sample are lysed, allowing released VASP to be captured by an anti-human VASP antibody which is coated in the microtiter plate. Then, a peroxidase-coupled anti-human VASP-P antibody binds to phosphorylated serine 239 antigenic determinant of VASP. The bound enzyme peroxidase is then revealed by its activity on TMB substrate over a predetermined time. After stopping the reaction, absorbance at 450 nm is directly related to the concentration of VASP-P contained in the sample. The VASP index was calculated using optical density (OD 450 nm) of samples incubated with PGE1 or PGE1 and ADP according to the formula: $VASP = [(OD450nm_{(PGE1)} - OD450nm_{(PGE1+ADP)})/OD450nm_{(PGE1)}] \times 100$. Optimal platelet reactivity inhibition was defined as a VASP index <50% according to the consensus document on the definition of high on-treatment platelet reactivity [14].

2.5. Clinical follow-up

All patients were followed in-hospital for ischemic and bleeding event. Ischemic events included death, myocardial infarction and urgent revascularization. Bleedings included BARC > 2.

2.6. Statistical analysis

Analyses were performed with the Graphpad Prism software v5.0 for windows (Graphpad Software Inc., San Diego, USA). All tests were two-sided and considered significant if <0.05. Categorical data are expressed as counts (%) and were compared using χ^2 or Fisher's exact tests. Continuous variables are expressed as median and interquartile range (IQR) and were compared were compared using the Mann Whitney test. A p value <0.05 is considered significant.

3. Results

3.1. Baseline characteristics of the population

Twenty patients were included and randomized to bivalirudin (n = 10) or heparin (n = 10). Their baseline characteristics were similar. The majority of patients were admitted for NSTEMI in both groups (p = 1). The baseline characteristics of the 2 groups are displayed in Table 1.

3.2. Angiographic characteristics

The numbers of diseased and treated vessels were similar between the 2 groups. Angiographic and interventional characteristics of the studied population are summarized in Table 2.

3.3. Platelet reactivity

The course of PR inhibition assessed by the VASP index was similar at all time points between the 2 groups. An optimal PR inhibition was obtained in all patients within 4 h (Fig. 1).

At baseline all patients had a VASP index >50% (median 88%; IQR: 74.5–96.5%; p = 0.7 between the 2 groups). Two hours after the 180 mg ticagrelor LD the median VASP index was 30% (IQR: 16.7–78.5%; p = 0.5 between the 2 groups). Finally 4 h after the loading dose, the median VASP index was 21% (IQR: 6–28%; p = 0.3). At 24 h post-LD the median VASP index was 9% (IQR: 5–18.2%) in the overall cohort (5 vs 9%; p = 0.5 between the 2 groups).

When considering optimal PR inhibition as a VASP index <50%, at 2 h post-LD 2 patients in the bivalirudin group did not achieve optimal PR inhibition compared to 4 in the heparin group (p = 0.3). At 4 h post-LD, 1 patient in the heparin group did not reach a VASP index <50% compared to none in the bivalirudin group (p = 1). Finally 24 h after the loading dose all patients had a VASP <50% in both groups.